

# Peptide Mapping of Adeno-Associated Virus Capsid Proteins

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## Overview

The use of Adeno-Associated Viruses (AAV) as an effective vector for the delivery of ssDNA in gene therapy, is well established and continues to grow.

As with any protein, viral proteins (VPs) are subject to post-translational modifications that can impact stability, capsid assembly, and overall efficacy. As a result, peptide mapping can be used to evaluate the presence and identification of these modifications.

At first glance AAVs present additional complexity not encountered with typical biologics. The viral capsid is a sixty-protein icosahedron comprised of three capsid, or viral proteins, non-covalently assembled in a ratio of 1:1:10 (VP1:VP2:VP3). However, a traditional peptide digest approach is sufficient to yield expected results. Although given the sequence homology, variation of signal intensity for peptides with overlapping and non-overlapping sequences should be anticipated.

In this application note, we profile capsid protein peptides from AAV5 and AAV9, digested with trypsin using a Biozen™ Peptide XB-C18 reversed phase column. We show the Biozen Peptide XB-C18 column provides excellent peak capacity and separation with a high level of sequence coverage.

## Sample Preparation

Step	Description
Sample:	20 µL AAV with concentration 2E13 vg/mL
Denaturation:	50 % v/v Trifluoroethanol
Reduction:	2 mM Final Tris (2-carboxyethyl) Phosphine
Incubation:	70 °C for 30 minutes
Alkylation:	2 mM Final Iodoacetimide
Incubation:	22 °C for 30 minutes, in the dark
Dilution:	4.5X with 100 mM Ammonium Bicarbonate, pH 8.25
Digestion:	1:25 w/w Trypsin
Incubation:	37 °C for 18 hours
Quench:	0.4 % v/v with 10 % Formic Acid
Concentration:	SpeedVac to approx. 25 % of final reaction volume
Resuspension:	Add equal volume of 25 mM Ammonium Bicarbonate, pH 8.25

## LC Conditions

**Column:** Biozen 2.6 µm Peptide XB-C18

**Dimension:** 150 x 2.1 mm

**Part No.:** OOF-4768-AN

**Mobile Phase:** A: 0.1 % Formic Acid in Water  
B: 0.1 % Formic Acid in Acetonitrile

Gradient:	Time (min)	%B
	0	2
	50.5	60
	55.5	60
	60	90
	63	90
	63.1	2
	70	2

**Flow Rate:** 0.3 mL/min

**Injection Volume:** 1 µL

**Temperature:** 60°C

**Instrument:** Vanquish™ UHPLC

**Detection:** MS

**Detector:** Q Exactive™ Orbitrap™ Plus

## MS Conditions

**Scan Type:** Full MS

**Resolution:** 70,000

**AGC Target:** 3e6 ms

**Maximum IT:** 200 ms

**Scan Range:** 200 to 2000 m/z

**Scan Type:** MS2

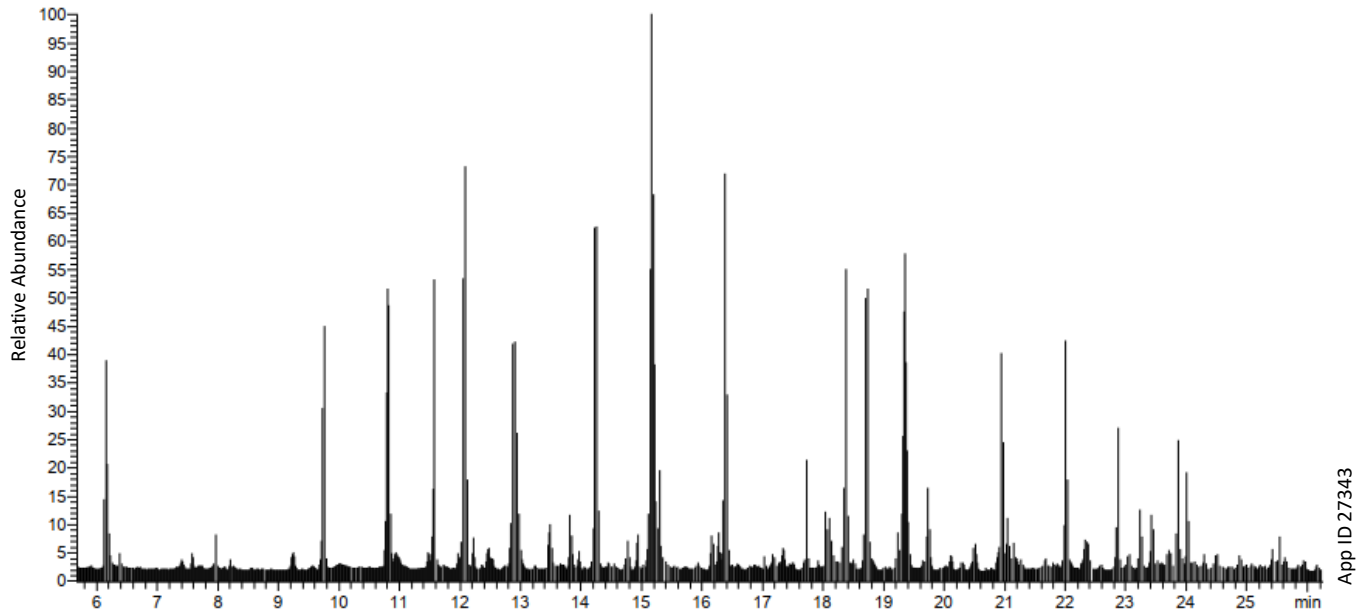
**Resolution:** 70,000

**AGC Target:** 1e5 ms

**Maximum IT:** 200 ms



Figure 1. Peptide Mapping of AAV5 VPs, at a Concentration of 6.7E12 vg/mL (Approx. 55 ng/μL).



Protein Name	# Peptides	% Coverage	# AAs
>tr Q9YIJ1 Q9YIJ1_9VIRU Capsid protein OS=adeno-associated virus 5 OX=82300 GN=cap PE=1 SV=1	193	93.6	723

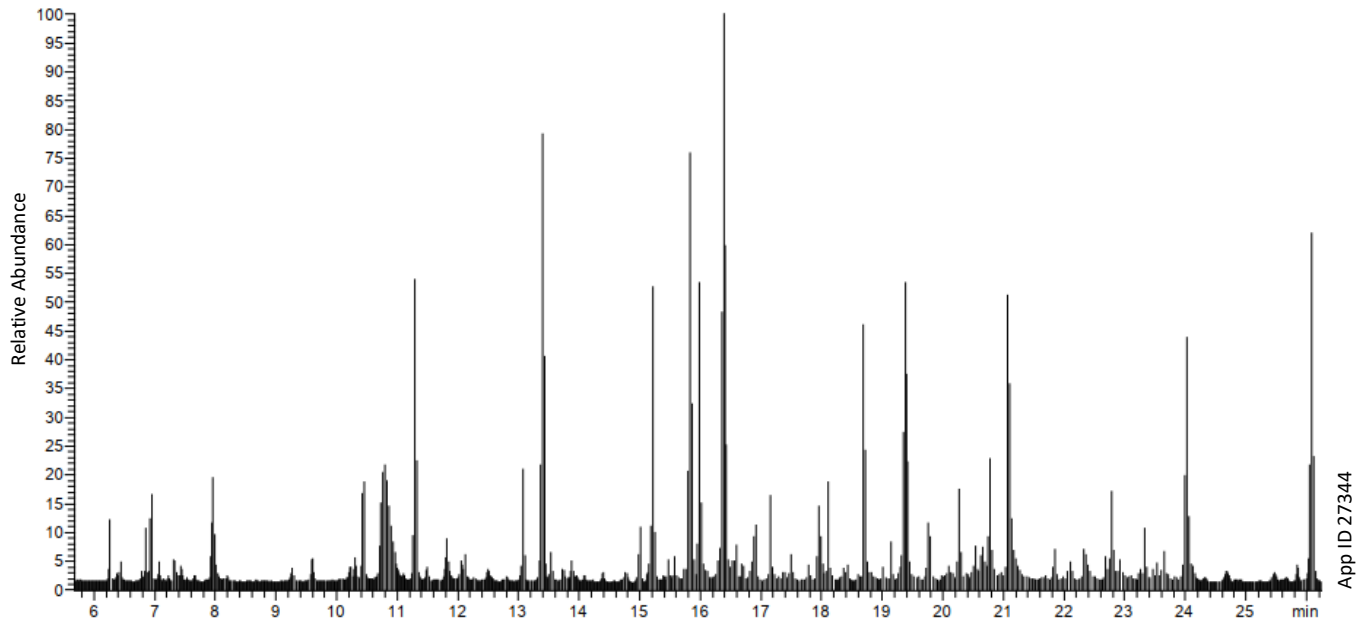
>tr|Q9YIJ1|Q9YIJ1\_9VIRU Capsid protein OS=adeno-associated virus 5 OX=82300 GN=cap PE=1 SV=1

```

10 20 30 40 50 60 70 80 90 100
SFVDHPPDWELEVGEGLREFLGLLEAGPPKPKPNQQHQDQARGLVLPGYNYLPGNGLDRGEPVNRADDEVAREHDSYNEQLEAGDNPYLKYNHADAQEFQE
110 120 130 140 150 160 170 180 190 200
KLADDTSFGGNLGKAVFOAKKRVLLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADTMSAGGGGPI
210 220 230 240 250 260 270 280 290 300
GDNNQGADGVGNASGDVHCDSTVMGDRVVTKSTRVVLPSYNNHQYREIKSGSVDGSMANAYFGYSTPWGYFDNRFHSHWSPRDQQLINNYWGFPRPS
310 320 330 340 350 360 370 380 390 400
LRVKIFNIQVKEVTVDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPOYGYATLNRDNTENPTERSFFCLEYFPSKMLRTGNN
410 420 430 440 450 460 470 480 490 500
FEFTYNFEEVPPFHSSFAPSONLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNVFPGPNGRTQGVNLSGSGVNRASVSAFATTNRMELEGAS
510 520 530 540 550 560 570 580 590 600
YQVPPQPNGHTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRYAVNVGGQMATNNQSSTTAPATGTYNLQEI VPGSVWNERDV
610 620 630 640 650 660 670 680 690 700
YLQGPVWAKIPETGAHFHPSFAMGGFGLKHPPPMLIKNTPVPGNITSFSDVPVSSFITQYSTQVTVEMEWELKKENSKRWNPETIQTNNYNDPQFVDF
710 720
APDSTGEYRTTRPIGTRYLTRPL
    
```



Figure 2. Peptide Mapping of AAV9 VPs, at a Concentration of 6.7E12 vg/mL (Approx. 55 ng/ $\mu$ L).



Protein Name	# Peptides	% Coverage	# AAs
>tr Q6JC40 Q6JC40_9VIRU Capsid protein VP1 OS=Adeno-associated virus 9 OX=235455 GN=cap PE=1 SV=1	205	92.7	768

```

>tr|Q6JC40|Q6JC40_9VIRU Capsid protein VP1 OS=Adeno-associated virus 9 OX=235455 GN=cap PE=1 SV=1
      10      20      30      40      50      60      70      80      90     100
MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEEF
QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAOPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGS
      210     220     230     240     250     260     270     280     290     300
LTMASGGGAPVADNNEGADGVSSSGNWHCDSQWLGDRVITTSTRTVALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR
      310     320     330     340     350     360     370     380     390     400
LINNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSAVGRSSFYCLEYF
      410     420     430     440     450     460     470     480     490     500
PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLNNPLIDQYLYLSKTINGSGQNQTLKFSVAGPSNMAVQGRNYIPGPSYRQRVSTTVTONNSE
      510     520     530     540     550     560     570     580     590     600
FAWPGASSVALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG
      610     620     630     640     650     660     670     680     690     700
ILPGVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGFGFGMKHPPPQILIKNTPVADPPTAFNKDKLNSFITQYSTGQVSVEIEVELQKENSKRVNPEIQ
      710     720     730     740     750     760
YTSNYYKSNNVEFAVNTEGVYSEPRIGYTRYLTRNLNYKSVNVDFTVDTNGVYSEPRIGYTRYLTRNL
    
```



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